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Roy, Krisna; Lyhs, Ulrike; Pedersen, Karl

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Inhibition studies of natural resin acids to *Clostridium perfringens* and *Escherichia coli* O149

Krisna Roy, Ulrike Lyhs and Karl Pedersen

National Veterinary Institute, Technical University of Denmark, Bülowsvej 27, 1870 Frederiksberg C, Copenhagen, Denmark

Introduction

As feed antibiotics are no longer allowed in Europe and some other countries, natural means to control the proliferation of pathogenic bacteria in the intestinal lumen of animals are needed. Natural resin acids are such natural products which have antimicrobial properties. In the present study, we aimed to evaluate the inhibitory activity of a resin-based product at different concentrations on intestinal bacterial pathogens.

Materials and methods

The targeted product was Progres® (Suomen Rehu) containing 8% resin acids. *Clostridium perfringens* isolated from chickens, turkeys and pigs, respectively, and *Escherichia coli* O149 from pigs were tested. Growth of the pathogens was tested at 0.01%, 0.1% and 0.5% concentrations of the product. Inhibitory bioactivity of the product was examined via OD_{600} measurements on growing cultures, by a 10-fold broth dilution method (DM), and by using an agar diffusion method (ADM). The OD method was followed only in one strain of *E. coli* O149. The DM was applied to three strains of each of the bacteria. Samples were taken after during incubation, where after 10-fold dilutions were made and plated onto blood agar plates. Counts were expressed as colony forming unit per ml (cfu/ml). The ADM was run on one strain of each of the bacteria, where zones of inhibition (mm) were measured. Subsequently, 10 *Cl. perfringens* strains, five from pigs, four from chickens and one from turkey, were tested with ADM against 0.5%, 1% and 5%.

Results

OD measurements were difficult to interpret due to a considerable contribution of the test product to the turbidity. Therefore c.f.u. measurements were considered more accurate. In DM, no *Cl. perfringens* was found at any concentration of the product, indicating an efficient inhibition of *Cl. perfringens*. At 0.1% and 0.5% of the product, there was apparently lower cfu/ml of two strains of *E. coli* O149 compared to the corresponding controls, but *E. coli* was considerably less inhibited than *Cl. perfringens*. In ADM, zone of inhibition (ZI) was evolved around the product-concentration of 0.5% (ZI: 8 to 10 mm), 1% (8.5 to 12.0 mm), and 5% (9.0 to 19.5 mm) when performed on all ten strains of *Cl. perfringens*. No strain of *E. coli* O149 was inhibited by the product at any concentration in ADM, and not at 0.01% in DM.

Conclusion

Cl. perfringens was inhibited even at low concentrations of the product containing resin acids, but there seemed to be some strain variation. *E. coli* O149 was only inhibited by high concentrations.